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Case Report

Hepatitis C Virus-Induced Leuko-Thrombocytopenia and Haemolysis

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Hepatitis C virus (HCV) has been recognized as the cause of thrombocytopenia occurring in patients with chronic hepatitis C, possibly through autoimmune mechanisms. A patient is described with B cell chronic lymphocytic leukaemia, presenting with a marked leuko-thrombocytopenia and an associated mild haemolysis secondary to HCV infection, in the absence of clinical and biochemical signs of hepatitis. Anti-HCV antibodies were detected in the serum both by ELISA and RIBA but not 2 months before the onset of cytopenia. The presence of HCV RNA was documented both in the peripheral blood mononuclear cells and in the bone marrow by reverse transcriptase polymerase chain reaction of the 5′ untranslated region of the viral genome. Of interest, HCV RNA was also found in the serum, showing that viraemia was associated with the presence of circulating anti-HCV antibodies. HCV genotyping, performed by PCR amplification of the core region, revealed the presence of an unclassifiable genotype. The hypothetical mechanisms leading to HCV-induced cytopenia in this patient are briefly discussed. Treatment with corticosteroids was effective in controlling blood cell counts, without increasing viraemia and deterioration of liver disease. HCV infection should be considered in the differential diagnosis of possible causes of cytopenia, mainly in immunosuppressed patients, even in absence of clinical and biochemical signs of hepatitis. ***J. Med. Virol. 53:182–184, 1997.***

KEY WORDS: anaemia; cytopenia; haemolysis; HCV; leukopenia; thrombocytopenia

INTRODUCTION

Viral agents have the ability to cause severe cytopenias by either a direct cytopathological effect or immunological mechanisms. Among these, parvovirus B19 can induce aplastic crises and has been involved in chronic marrow failure. Herpesviruses, such as cytomegalovirus (CMV) and human herpesvirus-6 (HHV-6), may cause serious bone marrow depression in immunocompromised hosts, representing a frequent cause of graft failure in marrow transplant patients. The human immunodeficiency virus (HIV) has been involved directly in the cytopenia occurring during the course of AIDS. Finally, a non-A, non-B, non-C hepatitis virus is likely to be the cause of the hepatitis-associated aplastic anaemia [Kurtzman and Young, 1989].

Hepatitis C virus (HCV) is related genetically to flaviviruses, which infect haematopoietic cells and are commonly responsible for neutropenia, marrow hypocellularity, and abnormal megakaryocytopoiesis [Kurtzman and Young, 1989]. Recently, thrombocytopenia has been documented in a significant percentage of patients with HCV chronic hepatitis with elevated platelet-associated immunoglobulin G (PAIgG), suggesting that chronic infection with HCV may produce a significant autoimmune reaction to platelets [Nagamine et al., 1996]. Furthermore, an association between HCV infection and idiopathic thrombocytopenic purpura has been recently proposed [Pollock, 1996; Pivetti et al., 1996].

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A case is described of thrombocytopenia in combination with leukopenia and haemolysis secondary to HCV infection, which responded to steroid treatment.

CLINICAL FINDINGS AND TEST RESULTS

A 72-year-old man with B cell chronic lymphocytic leukaemia (B-CLL) since December 1988, has been maintained in complete remission by intermittent chemotherapy until August 1995. Since February 1994, the patient has been receiving immunoglobulin treatment (30 g monthly), because of a markedly low level of serum immunoglobulins. In August 1995 a marked leuko-thrombocytopenia (white blood cell counts (WBC) 1.9 × 10^9/l, with 50% lymphocytes, platelets (Plts) 23.0 × 10^9/l) and moderate anaemia (haemoglobin, Hb, 10.2 g/l) appeared. The bone marrow examination showed a slightly increased haematopoiesis with a lymphocyte infiltration of less than 10%, with no signs of haemophagocytosis. At that time, anti-HCV antibodies were detected, for the first time, both by enzyme-linked immunosorbent assay (ELISA) and recombinat immunoblot assay (RIBA) [Luppi et al., 1996]. It should be noted that serum anti-HCV antibodies were not detected, by repeated ELISA and RIBA, the last of which were carried out in May 1994. HCV RNA sequences were identified both in the peripheral blood mononuclear cells (PBMCs) and in the bone marrow by reverse transcriptase polymerase chain reaction (RT-PCR) of the 5′ untranslated region (5′ UTR) of the viral genome [Luppi et al., 1996]. More importantly, viral sequences were also detected in the serum, showing that HCV viraemia was associated with circulating anti-HCV antibodies. HCV genotyping, carried out by PCR amplification of the core region according to the method described by Okamoto et al. [1993], with the modifications described by Zignego et al. [1996], showed the absence of the most common genotypes (I,II,III,IV,V, corresponding to 1a,1b,2a,2b,3a, of the Simmonds classification), and the presence of an unclassifiable genotype [Luppi et al., 1996]. Serology for common herpesviral infections, including Epstein-Barr virus, CMV, HHV-6, as well as HIV 1 and 2 was consistently negative. Coagulation screen and routine blood chemistry values were normal, and there were neither clinical nor biochemical signs of hepatitis. Of interest, abnormally elevated PAIgG levels were observed. Serum haptoglobin was undetectable, while the Coomb's test was repeatedly negative. Tests for anti-nuclear antibody (ANA) and anti-DNA as well as rheumatoid factor (RF) and C reactive protein (CRP) were negative. Cold-agglutinins and cryoglobulins could not be detected. Treatment with prednisone (25 mg/day) was started, resulting in an increase of blood cells (WBC 6.4 × 10^9/l, Plts 63.4 × 10^9/l, and Hb value 11.9 g/l). However, it should be noted that a reduction of daily dose of prednisone (12.5 mg) resulted in a fall of cell counts, so that a higher dose of steroid was restored (Fig. 1). In October 1995, progression of CLL was documented and remission was achieved after five courses of chemotherapy, including cyclophosphamide (Cy) and vincristine (Vcr) plus prednisone. During this period, the WBC and the Plt counts were maintained at about 4.5 × 10^9/l and 45.0 × 10^9/l, respectively (Fig. 1). At present, a dose of 25 mg/day prednisone is necessary to prevent reduction in blood cell counts.

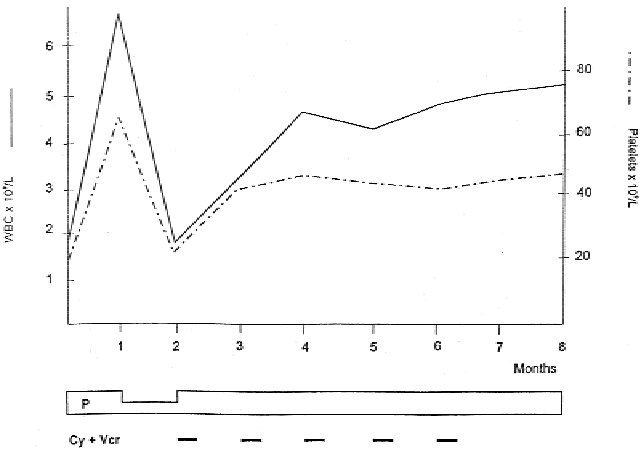

Fig. 1. Correlation between WBC and platelet counts and therapy with prednisone (P) and cyclophosphamide plus vincristine (Cy + Vcr). P: 25 mg/day; 12.5 mg/day during the 2nd month.

DISCUSSION

The haematological abnormalities in this patient were secondary to the occurrence of HCV viraemia, demonstrated both by serological and molecular testing. HCV has a tropism for haemopoietic cells in vivo and HCV proteins as well as HCV RNA genomic sequences were detected both in bone marrow and peripheral blood mononuclear cells of chronically HCV-infected patients [Sansonno et al., 1996]. However, a direct cytopathological effect does not seem to have occurred in this patient, since routine bone marrow examination showed normal cellularity. Similarly, virus-induced haemophagocytosis can be ruled out. Thus, the mechanism for the cytopenia secondary to HCV infection in this patient is likely to be immunological, as suggested by the effectiveness of immunosuppressive drugs in controlling blood cell counts. Thrombocytopenia is likely to be mediated by the appearance of PAIgG, as it often occurs in HCV infected patients [Nagamine et al., 1996], although it is not known whether there is a strong homology between the HCV proteins and platelet protein components. Relevant to this, serum anti-HCV antibodies have been detected in 19% of 112 patients with autoimmune thrombocytopenic purpura [Pollock, 1996]. An even higher prevalence of HCV infection (36.4%) has also been reported in another series of patients with idiopathic thrombocytopenic purpura [Pivetti et al., 1996]. Unexpectedly, the mild haemolysis in our patient is not associated with the presence of positive direct antiglobulin test, but the occurrence of a Coomb's-negative haemolytic anaemia in combination with thrombocytopenia, had been reported during acute CMV infection (van Spronsen et

al., 1996). Our patient did not present marrow failure nor hypersplenism, which represent frequent causes of cytopenia in patients with advanced-stage CLL. However, cytopenias may also occur as autoimmune phenomena in CLL, but while autoimmune, Coomb's-positive, haemolytic anaemia may occur in 7–35% of cases, during the course of the disease, autoimmune thrombocytopenia and leukopenia are less common and more difficult to confirm [Faguet, 1994]. It should be noted that this patient had never manifested autoimmune complications before and the disease was in clinical remission at the onset of the cytopenia. Thus, it is unlikely that the leuko-thrombocytopenia and Coomb's-negative haemolysis in this patient are related to the underlying lymphoproliferative disease, although the possibility exists that the triggering of immunological abnormalities by HCV infection has been favoured by a susceptibility to autoimmune phenomena which is characteristic of a number of patients with B-CLL.

HCV infection is associated with autoimmune diseases, including autoimmune hepatitis but also several extrahepatic immunologic manifestations, such as type II mixed cryoglobulinaemia, membranoproliferative glomerulonephritis, autoimmune thyroiditis, and salivary gland lesions resembling Sjögren's syndrome [Pawlotsky et al., 1995]. The description of our case suggests that HCV may induce cytopenia, possibly acting through immunological mechanisms. Recently, it has been suggested that HCV genotype III (2a) is more frequently detectable in patients with mixed cryoglobulinaemia and circulating autoantibodies, and thus may be involved in the pathogenesis of autoimmune-lymphoproliferative disorders [Zignego et al., 1996]. In our patient, genotype could not be classified.

It has been shown recently that immunosuppressive drugs, such as prednisone and azathioprine are useful for patients with combined features of both autoimmune hepatitis and chronic hepatitis C, suggesting that the risk for increasing HCV replication and deterioration of the liver disease under immunosuppression may be substantially less than expected [Bellary et al., 1995]. In some cases, the immunosuppression induced by chemotherapy is required to control immune-mediated liver damage associated with HCV infection, and the occurrence of fulminant hepatitis following withdrawal of chemotherapy has been reported in two carriers of HCV with lymphomas [Vento et al., 1996]. In our patient the degree of viraemia has remained stable and routine biochemical liver function tests, have remained normal, despite immunosuppressive therapy. Testing for serological and molecular markers of active HCV infection is recommended, mainly in patients with lymphoproliferative disorders and immune impairment, since recognition of HCV infection may also have therapeutic implications.

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